These amendments are made without prejudice and are not to be construed as abandonment of the previously claimed subject matter or agreement with the Examiner's position. In accordance with the requirements of 37 C.F.R. § 1.121, a marked up version of the claims showing the changes, is attached as Appendix A. For the Examiner's convenience, a complete set of the currently pending claims is also provided as Appendix B.

REMARKS

Status of the Claims.

Claims 1, 3-21 and 23-25 are pending in the application, claims 2 and 22 being canceled with the amendment, and claims 23-25 being added. Claim 1 has been amended to incorporate an element previously recited in claim 2 and to delete non-essential elements, which are now recited in claim 23. Claim 6 has been amended to add the term "cosmid." Support for this amendment can be found in the specification at least at page 10, line 3. Claims 14, 16-18, and 20 have been amended to correct claim dependencies in light of the cancellation of claim 2. Claim 21 has been amended to incorporate an element previously recited in claim 22. Claim 24 is analogous to claim 23, but depends from claim 21 Claim 25 finds support in the specification at least at page 10, lines 7-9. The amendments add no new matter.

Under 37 C.F.R. § 1.116, amendments to the claims after final rejection may be entered if the amendments place the claims in better form for consideration on appeal. Applicants submit that the above amendments meet this criterion. In addition, § 1.116 states that amendments cancelling claims may be entered after final rejection. These amendments are necessary to more clearly recite Applicants' invention. The amendments were not presented earlier because the need for the amendments was not apparent until the Final Office Action was received. Entry of the amendments is thus permitted under § 1.116 and is respectfully requested.

35 U.S.C. § 112, Second Paragraph.

Claims 1-22 were rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. Office Action, page 2. The rejection is respectfully traversed.

The rejection was based, in part, on the Examiner's view that the phrase "the corresponding first polynucleotide" lacked antecedent basis and was unclear. *Id.* Applicants submit that the recitation in claim 1(a) that "each sample is derived from a first polynucleotide" provides

clear antecedent basis for "the corresponding first polynucleotide." Moreover, Applicants submit that one of skill in the art would have had no difficulty understanding that the phrase "resuspending each amplification product to form a target solution representative of the corresponding first polynucleotide" referred to a target solution that is representative of the first polynucleotide from which the amplification product was derived. This element has been moved to claim 23, and claim 1 has been amended to recite "forming a target solution for each sample, wherein each amplification product is representative of the first polynucleotide corresponding to each sample," which Applicants submit is equally clear. Applicants note that this amendment does not narrow claim 1 and thus cannot be construed as an abandonment of any subject matter.

The only other claim that includes the term "corresponding" is claim 21(c). As the language of this element is the same as that of claim 1(c), Applicants submit that claim 21(c) is clear and definite for at least the same reasons as claim 1.

The Examiner also based the rejection on the view that "it is not clear as to how one determines whether or not a target solutions is representative of the corresponding first polynucleotide." *Id.* Applicants submit that one of skill in the art would readily understand when a target solution is representative of a polynucleotide in light of the definition of the term "representative" appearing in Applicants' specification at page 7, lines 7-15. Specifically, Applicants' specification states:

> A polynucleotide product is said to be "representative" of a starting polynucleotide if the hybridization signal observed from the polynucleotide product is sufficiently similar to that observed from the starting polynucleotide that the polynucleotide product can be substituted for the starting polynucleotide in a hybridization assay. In other words, a representative polynucleotide product performs essentially equivalently to the starting polynucleotide in a hybridization assay of interest.
>
> An array of polynucleotides is said to be "representative" of a collection of starting polynucleotides if the polynucleotides present in each target element are representative of the corresponding starting polynucleotide.

One of skill in the art readily appreciates that the target solution recited in claim 1 is "representative of the corresponding first polynucleotide" by virtue of the recited amplification product, which is, of course, a polynucleotide product. Accordingly, one of skill in the art would understand that an

amplification product representative of the corresponding first polynucleotide is one that can be substituted for the corresponding first polynucleotide in a hybridization assay.

As the metes and bounds of the pending claims would be clear to one of skill in the art, Applicants submit that the claims are clear and definite. Withdrawal of the § 112, second paragraph, rejection is respectfully requested.

35 U.S.C. § 102.

Claims 1, 3-13, and 21 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Smith (PCR Methods and Applications (1992) 2:21-27. Office Action, page 3. The rejection is respectfully traversed.

Claim 1 recites a "method for preparing an array of polynucleotides that is representative of a plurality of first polynucleotides." Claim 1 has been amended to incorporate the element recited in claim 2, namely that the target solutions are applied "to one or more substrates . . . to produce an array of polynucleotides."

The Examiner states that "Smith teaches ligation-mediated PCR of restriction fragments from large DNA molecules." Office Action, page 3. The Examiner believes that "it is a property of Smith's target solution comprising amplification products, that this target solution would be suitable for application to a substrate" *Id.*, page 4. However, the Examiner correctly states that "Smith does not teach using amplification products to generate an array" *Id.*, page 7. Presumably for this reason, claim 2, which recited this element, was not rejected under § 102 over Smith. Applicants therefore submit that the § 102 rejection of claim 1 is moot in light of the amendment of claim 1 to recite "applying target solutions comprising the amplification products to one or more substrates . . . to produce an array of polynucleotides," which previously appeared in claim 2. Claims 3-13 depend, directly or indirectly, from claim 1 and are thus free of the § 102 rejection over Smith for at least the same reason as claim 1.

Turning to the § 102 rejection of claim 21, this claim relates to a plurality of target solutions prepared according to the method recited in originally filed claim 3. Claim 21 has been amended to explicitly recite elements of claim 3. In addition, claim 21 has been amended to incorporate the element recited in originally filed claim 22, namely that "the target solutions comprise dimethyl sulfoxide at a concentration of about 20% by volume." Smith neither teaches nor suggests target solutions comprising DMSO at this concentration, which is presumably why the

Examiner did not include claim 21 in the § 102 rejection over Smith. Accordingly, Applicants submit that the § 102 rejection of claim 21 is moot in light of the amendment of claim 21 to recite that "the target solutions comprise dimethyl sulfoxide at a concentration of about 20% by volume," which previously appeared in claim 22.

As claims 1, 3-13, and 21 are clearly distinct from the methods and compositions described in Smith, Applicants respectfully request withdrawal of the § 102 rejection.

35 U.S.C. § 103(a).

Smith and Brown

Claims 2, 14-16, and 20 stand rejected under 35 U.S.C. § 103(a) as allegedly obvious in light of Smith in view of Brown et al. (USPN 5,807,522). Office Action, page 6. This rejection is respectfully traversed.

The element recited in claim 2 has been incorporated into its base claim, claim 1, and claim 2 has been canceled. Accordingly, the § 103 rejection over Smith and Brown is addressed herein with respect to claim 1. The method of claim 1 employs "a plurality of samples of double-stranded polynucleotide fragments, wherein each sample is derived from a first [i.e., a starting] polynucleotide." According to the method, each sample is amplified to form an amplification product that is "representative of the corresponding first polynucleotide." Target solutions containing the amplification products are then applied to one or more substrates to produce an array of polynucleotides that is representative of the plurality of first polynucleotides. It is important to note that the specifically recited amplification steps consistently produce amplification products that are highly representative of the starting polynucleotides. This feature of the invention facilitates quantitative comparison among hybridization signals produced when the target solutions are arrayed and hybridized with sample polynucleotides. Claims 14-16 depend, directly or indirectly, from claim 1. Claim 20 recites an "array of polynucleotides that is representative of a plurality of first polynucleotides."

The three elements of a *prima facie* case of obviousness are: (1) the reference(s) must teach or suggest all of the elements of the claimed invention, (2) there must some motivation for combining or modifying the teachings of the references to arrive at the claimed invention, and (3) the reference(s) or knowledge in the art must provide a reasonable expectation of success, i.e., a reasonable assurance that the claimed invention would work.

Applicants submit that the Examiner has failed to establish any of the elements of a *prima facie* case of obviousness. First, neither Smith, nor Brown, teach or suggest all of the elements of independent claims 1 or 20. Specifically, neither Smith, nor Brown, teach or suggest producing and arraying target solutions that are as highly representative of a plurality of first polynucleotides as that produced by the method steps recited in claim 1 and incorporated into 20.

As described in the specification, representative target solutions are produced by first fragmenting a starting polynucleotide. Applicants' specification, page 10, lines 14-25. Applicants' specification states:

> Adaptors are ligated to each end of the polynucleotide fragments, which provides the fragments with common priming sites for amplification. Adaptors are typically designed to serve as efficient amplification primers so that unligated strands of the adaptors can be employed to amplify the sequences between the priming sites. This approach allows amplification of a polynucleotide without prior knowledge of the nucleotide sequence and allows the production of amplification products that are representative of the starting polynucleotide . . . .

Id. These method steps ***amplify essentially all of the polynucleotide sequences in the mixture*** produced by fragmenting the starting polynucleotide. Furthermore, amplification is carried out, according to the claimed method, to ***amplify the polynucleotide sequences in the mixture to essentially the same extent***. These features of the invention are incorporated into the method steps of claim 1 and in the recitation in claims 1 and 20 of "an array of polynucleotides that is representative of a plurality of first [*i.e.*, starting] polynucleotides." The recited method steps yield an amplification product that, when hybridized, for example with total DNA, produces a hybridization signal sufficiently similar to that observed from the starting polynucleotide that the amplification product can be substituted for the starting polynucleotide in a hybridization assay. In other words, the starting polynucleotide sequences are present in the amplification product in approximately the same proportions as in the starting polynucleotide.

By contrast, Smith discloses a "general method . . . for PCR amplification of ***single restriction fragments*** from large DNA molecules." Smith, abstract (emphasis added). Smith emphasizes the importance of the "ability to generate ***unique*** amplified fragments." Smith, page 23, col. 2. The experimental work disclosed in this article entailed the amplification of specific restriction fragments from restriction digested lambda DNA. *See, e.g.*, Smith, page 22, col. 3 and

Fig. 2 (showing that amplification produced a single 163 bp fragment). In summing up, Smith states: "This study demonstrates the applicability of ligation-mediated PCR to amplifying ***individual*** type IIS restriction fragments from large DNA molecules." Smith, page 24, col. 3 (emphasis added).

The Smith method seeks to amplify a specific fragment of a large DNA molecule, such as lambda DNA. This goal is diametrically opposed to that of the claimed method, which is to produce an amplification product that is representative of the entire starting polynucleotide, not just a fragment. Thus, Smith's teaching regarding the amplification of a unique sequence within a larger molecule teaches directly away from the claimed method and compositions.

In response to this argument, which Applicants made previously, the Examiner stated:

> This argument is not persuasive, as Applicants' [*sic*] are arguing limitations not found in the claims. The claims only require that the target solution is "representative of the corresponding first polynucleotide", and therefore, the claims do not specify that the target solutions <u>must</u> be representative of the "entire" starting polynucleotide, not just a fragment.

Office Action, page 5. This statement overlooks the fact that the recited method steps enhance the production of

target solutions in which the starting polynucleotide sequences are present in approximately the same proportions as in the starting polynucleotide prior to amplification. Claim 1, for example, recites:

> a) providing a plurality of samples of double-stranded polynucleotide fragments, wherein each sample is derived from a first polynucleotide;
>
> b) ligating adapters to each end of the polynucleotide fragments of each sample to produce modified polynucleotide fragments, wherein each adapter comprises a first strand and a second strand, the second strand having a region of substantial complementarity to a region of the first strand;
>
> c) using sequences within the adapters to amplify the modified polynucleotide fragments to produce an amplification product for each sample of polynucleotide fragments, wherein each amplification product is representative of the first polynucleotide corresponding to each sample

Steps (a) and (b) add universal priming sites to either end of essentially all polynucleotide fragments in each sample mixture. In step (c), the universal primer sites are used to amplify essentially all of

the modified polynucleotide fragments. Thus, the recited method steps necessarily produce target solution that must be representative of essentially the entire starting polynucleotide. One of skill in the art would readily appreciate the essentially proportionate amplification provided by the recited method steps is completely different from the selective amplification of sequences corresponding to a single specific fragment of the starting polynucleotide. Furthermore, one of skill in the art would understand that, unlike the amplification product recited in the claim, a selectively amplified fragment of a starting polynucleotide does not perform equivalently to the starting polynucleotide in a hybridization assay because only a probe specific for the particular fragment can be used for detection.

The concept of "essentially equivalent performance" in a hybridization assay is illustrated in Figure 1 and discussed in the brief description of Figure 1, as follows:

> Fig. 1 shows the results of comparative genomic hybridization ("CGH") of DNA from the breast cancer cell line BT474 (labeled with FITC-dCTP) and normal female DNA (labeled with Cy3-dCTP) to an array containing target elements prepared from BAC clones containing chromosome 20 sequences using the methods of the invention. The ratio of the BT474 DNA:normal DNA hybridization signal (normalized ratio) is shown for amplification products prepared from BAC clones using ligation-mediated PCR (PCR1-3), as compared to historical data from an array of BAC DNA that was isolated conventionally. Three independently prepared amplification products were produced for most of the BAC clones that were amplified. These results demonstrate that ligation-mediated PCR produces an amplification product that is highly representative of (i.e., performs equivalently to) the BAC clone that serves as the template.

Applicants' specification, page 5, lines 9-19.

Thus, Applicants' invention is aimed at fragmenting a starting polynucleotide and amplifying essentially all of the polynucleotide sequences in the resulting mixture, to essentially the same extent, which produces an amplification product (and, ultimately, target solution) wherein the starting polynucleotide sequences are present in approximately the same proportions as in the starting polynucleotide. Accordingly, the claimed invention requires the production of a target solution that is representative of essentially an entire starting polynucleotide. Smith, on the other hand, teaches selective "amplification of single restriction fragments from large DNA molecules." If the recited amplification products and those disclosed in Smith were subjected to gel electrophoresis, followed by DNA staining, the recited amplification products would appear as a "smear," whereas

Smith's amplification products would appear as discrete bands. *See* Smith, Figs. 2-4. In teaching selective amplification of a particular restriction fragment of a starting polynucleotide, Smith teaches directly away from the target solutions of the invention.

Smith discusses the simultaneous amplification of multiple polynucleotide fragments in connection with multiplex DNA sequencing. However, as discussed in the previous Amendment, Smith expresses doubt about the practicability of this strategy. Smith, page 25-26. Applicants therefore submit that this disclosure would be unlikely to prompt one of skill in the art to do anything, much less to do anything like producing an amplification product that is representative of the corresponding starting polynucleotide.

The Examiner rejected this point on the ground that "Smith (pg. 26, col. 2) teaches that other possible applications of his method include fingerprinting purified cosmids, YACs, or even bacterial genomes, and multiplex chromosome walking in clone libraries arrayed in high density grids." Office Action, pages 5-6. However, nothing in the sentence on which the Examiner relies teaches or suggests that the amplified probe should be representative of the entire cosmid, YAC, or bacterial genome.

Thus, Smith fails to teach or suggest a "method for preparing an array of polynucleotides that is representative of a plurality of first polynucleotides" (claim 1) and a polynucleotide array having this feature (claim 20). Applicants submit that Brown, whether taken alone or in combination with Smith, fails to remedy this deficiency.

Brown is primarily concerned, not with the production of target solutions, but, as the Examiner recognizes, with a method and apparatus for applying target solutions to a substrate to fabricate microarrays. Office Action, page 4; Brown, abstract. Brown discloses the use of PCR to "randomly" amplify DNA for robotic spotting on substrates. Brown, col. 16, lines 9-22; col. 17, lines 46-55. However, nothing in Brown teaches or suggests any measures that would produce an amplification product (and, ultimately, target solution) wherein the starting polynucleotide sequences are present in approximately the same proportions as in the starting polynucleotide and thus representative of essentially the entire starting polynucleotide. Indeed, as discussed in the previous Amendment, Brown's reference to "random" PCR amplification indicates that random primers were used, which would not yield amplification products that were as highly representative of the starting polynucleotide as those produced according to the recited method steps.

Thus, the Smith-Brown combination fails to teach or suggest the amplification steps of claim 1, which result in proportionate amplification of essentially all starting polynucleotide sequences, much less, the arraying of target solutions containing amplification products produced in this manner. Accordingly, this combination fails to teach or suggest all of the elements recited in claims 1 and 20.

Turning to the second element of a *prima facie* case, Applicants submit that the record is devoid of any motivation to modify the Smith-Brown combination to produce the invention recited in claim 1 or in claim 20. Specifically, the Examiner has not demonstrated that the references would have provided motivation to those of ordinary skill in the art to combine or modify the elements that are disclosed to arrive at the claimed invention. A recent Federal Circuit case emphasizes that the requirement for specific motivation for combining or, as in the present application, modifying references is to be applied rigorously. The court stated in *In Re Werner Kotzab*, 217 F.3d 1365; 2000 U.S. App. LEXIS 15504; 55 USPQ2d 1313 (Fed. Cir. 2000):

> A critical step in analyzing the patentability of claims pursuant to section 103(a) is casting the mind back to the time of invention, to consider the thinking of one of ordinary skill in the art, guided only by the prior art references and the then-accepted wisdom in the field. *See Dembiczak*, 175 F.3d at 999, 50 U.S.P.Q.2D (BNA) at 1617. Close adherence to this methodology is especially important in cases where the very ease with which the invention can be understood may prompt one "to fall victim to the insidious effect of a hindsight syndrome wherein that which only the invention taught is used against its teacher." *Id.* (quoting *W.L. Gore & Assocs., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1553, 220 U.S.P.Q. (BNA) 303, 313 (Fed. Cir. 1983)).
>
> Most if not all inventions arise from a combination of old elements. *See In re Rouffet*, 149 F.3d 1350, 1357, 47 U.S.P.Q.2D (BNA) 1453, 1457 (Fed. Cir. 1998). Thus, every element of a claimed invention may often be found in the prior art. *See id.* However, identification in the prior art of each individual part claimed is insufficient to defeat patentability of the whole claimed invention. See *id.* Rather, to establish obviousness based on a combination of the elements disclosed in the prior art, there must be some motivation, suggestion or teaching of the desirability of making the specific combination that was made by the applicant. See *In re Dance*, 160 F.3d 1339, 1343, 48 U.S.P.Q.2D (BNA) 1635, 1637 (Fed. Cir. 1998); In re Gordon, 733 F.2d 900, 902, 221 U.S.P.Q. (BNA) 1125, 1127 (Fed. Cir. 1984). Even when obviousness is based on a single prior art reference, there must be a showing of a suggestion or motivation to modify the teachings of that reference. *See B.F. Goodrich Co. v. Aircraft Braking*

> *Sys. Corp.*, 72 F.3d 1577, 1582, 37 U.S.P.Q.2D (BNA) 1314, 1318 (Fed. Cir. 1996).

In Re Werner Kotzab, at 1369-1370. The Federal Circuit further stated that ***the particular finding required must explain why "the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed."*** *Id.* at 1371 (emphasis added).

The Examiner urges that one of skill in the art would have been motivated "to have further modified the method of Smith so to as to have applied the target solution comprising the amplified fragments to the microarray of Brown in order to have achieved the benefits of producing an array useful for fingerprinting large molecules such as YACs, cosmids, and bacterial genomes by DNA sequencing and gene expression analysis." Office Action, page 8. However, as pointed out above, one of skill in the art would, at best, be motivated by Smith to selectively amplify a single restriction fragment from a large DNA molecule, label the fragment, and use it to probe a DNA microarray, such as described in Brown. The Office Action fails to show that Smith and/or Brown recognized the technical difficulties associated with producing an array of polynucleotides that is as highly representative of a plurality of first polynucleotides as that produced according to the method of claim 1. Moreover the Office Action fails to demonstrated that Smith and/or Brown provided any hint of the specific solution to these difficulties that is recited in claim 1. As the record fails to provide any motivation for modifying the teachings of Smith and Brown to produce the method of claim 1 or the array of claim 20, the Examiner has failed to establish the second, as well as the first, requirement of a *prima facie* case of obviousness.

In addition, the Office Action does not address the third requirement for a *prima facie* case of obviousness, *i.e.*, whether one skilled in the art would have had a reasonable expectation of success in making the claimed invention based on the combined teachings of Smith and Brown. In particular, the record fails to demonstrate that one of skill in the art would have had a reasonable expectation of success in using the methods of Smith and Brown to produce an array of polynucleotides that is as highly representative of a plurality of first polynucleotides as the array of the present invention. One could not reasonably conclude that modifying Smith's selective amplification method in an effort to achieve proportionate amplification of all starting polynucleotide sequences, followed by Brown's microarray fabrication methods, would necessarily produce an array of polynucleotides wherein each target element of the array is representative of

(i.e., performs essentially equivalently to) the starting polynucleotide that served as the amplification template. For this additional reason, therefore, Applicants submit that the Examiner has failed to establish a *prima facie* case of obviousness over Smith in view of Brown.

Withdrawal of the § 103 rejection of claims 2, 14-16, and 20 is therefore respectfully requested.

Smith, Brown, and Gordon

Claim 17 was rejected under 35 U.S.C. § 103(a) as allegedly obvious in light of Smith in view of Brown and further in view of Gordon et al. (USPN 5,601,980). Office Action, page 9. The rejection is respectfully traversed.

Claim 17 depends ultimately from claim 1 and recites a method "wherein the target solutions are robotically spotted on the substrate." Gordon was cited for the teaching of robotic spotting. *Id.* However, Gordon fails to teach or suggest the amplification steps of claim 1, which result in approximately proportionate amplification of essentially all starting polynucleotide sequences. Moreover, Gordon is devoid of any teaching regarding the arraying of target solutions containing amplification products produced in this manner. Accordingly, the combination of Smith, Brown, and Gordon does not teach or suggest all of the elements of the claim1, which are incorporated into claim 17 by virtue of its dependence on claim 1. Gordon also does nothing toward establishing any motivation to modify the teachings of Smith and Brown to produce the method of claim 17, much less any expectation of success in doing so.

Because the Examiner has not established any element of a *prima facie* case of obviousness of claim 17 over Smith, Brown, and Gordon, Applicants respectfully request withdrawal of the 103 rejection of claim 17.

Smith, Brown, and Stimpson

Claim 18 was rejected under 35 U.S.C. § 103(a) as allegedly obvious in light of Smith in view of Brown and further in view of Stimpson et al. (Proc. Natl. Acad. Sci. USA (1995) 92:6379-6383). Office Action, page 10. The rejection is respectfully traversed.

Claim 18 depends ultimately from claim 1, which recites the use of adaptors in the claimed amplification reaction. Claim 18 recites that "at least one strand of the adapters includes an amino group." Stimpson was cited as teaching "DNA chips (i.e., array[s]), which are constructed by using 3'-amino-labeled oligonucleotides." Office Action, page 10. However, Stimpson discloses

that "DNA chips . . . were constructed by using ***presynthesized*** 3'-amine-labeled oligonucleotides." Stimpson, page 6380, col. 1. Stimpson thus fails to teach or suggest anything regarding any amplification-based method for producing target solutions for an array, much less the specific amplification steps recited in claim 1. Stimpson thus does nothing to remedy the above-noted deficiencies of Smith and Brown. Accordingly, the combination of Smith, Brown, and Stimpson does not teach or suggest all of the elements of the claim1, which are incorporated into claim 18. Stimpson also fails to provide any motivation to modify the teachings of Smith and Brown to produce the method of claim 18, much less any expectation of success in doing so.

Because the Examiner has not established any element of a *prima facie* case of obviousness of claim 18 over Smith, Brown, and Stimpson, Applicants respectfully request withdrawal of the § 103 rejection of claim 18.

Smith and Cronin

Claims 19 and 22 were rejected under 35 U.S.C. § 103(a) as allegedly obvious in light of Smith in view of Cronin et al. (WO 97/43450). Office Action, page 11. The rejection is respectfully traversed as to claim 19. The rejection is moot as to claim 22, which has been canceled.

Claim 19 depends from claim 1 and recites a method "wherein the target solutions comprise dimethyl sulfoxide [DMSO] at a concentration of about 20% by volume." Cronin was cited as teaching target solutions containing DMSO. Office Action, page 12. However, like the other references of record, Cronin fails to teach or suggest the amplification steps of claim 1, which result in approximately proportionate amplification of essentially all starting polynucleotide sequences. Furthermore, Cronin is devoid of any teaching regarding the arraying of target solutions containing amplification products produced in this manner. Cronin, like Stimpson, discloses arrays of synthetically produced oligonucleotides. Cronin, page 9, line 15 – page 10, line 29.

Cronin also discusses the polynucleotides to be analyzed by hybridization to the array, which Cronin calls "target" polynucleotides. Cronin, page 8, lines 6-7. It should be noted that Cronin's target polynucleotides are the sample polynucleotides that bind to array elements, whereas the polynucleotides in the target solutions recited in the pending claims are useful as array elements. Cronin describes amplifying target (i.e., sample) polynucleotides by "PCR." But this teaching of regarding the preparation of sample polynucleotides says nothing about the preparation of polynucleotides useful as array elements.

Thus, the Smith-Cronin combination fails teach or suggest all of the elements of the claim 1, which incorporated into claim 19. Cronin also fails to provide any motivation to modify the teachings of Smith to produce the method of claim 19 and further provides no expectation of success in doing so.

Because the Examiner has not established any element of a *prima facie* case of obviousness of claim 19 over Smith in view of Cronin, Applicants therefore respectfully request withdrawal of the § 103 rejection of claim 19.

Conclusion

In view of the foregoing, Applicants believe that all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (510) 769-3509.

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<u>APPENDIX A</u>

<u>"MARKED UP" CLAIMS ILLUSTRATING THE AMENDMENTS MADE TO THE CLAIMS OF 09/574,386 WITH ENTRY OF THIS AMENDMENT</u>

Amended claim(s):

1. (Twice Amended) A method for preparing [amplification products useful for forming] an array of polynucleotides that is representative of a plurality of first polynucleotides comprising:

a) providing a plurality of samples of double-stranded polynucleotide fragments, wherein each sample is derived from a first polynucleotide;

b) ligating adapters to each end of the polynucleotide fragments <u>of each sample</u> to produce modified polynucleotide fragments, wherein each adapter comprises a first strand and a second strand, the second strand having a region of substantial complementarity to a region of the first strand;

c) <u>using sequences within the adapters to amplify</u> [amplifying] the modified polynucleotide fragments to produce an amplification product for each sample of polynucleotide fragments<u>, wherein each amplification product is representative of the first polynucleotide corresponding to each sample</u>; <u>and</u>

[d) isolating each amplification product; and

resuspending each amplification product to form a target solution representative of the corresponding first polynucleotide, wherein the target solution is suitable for application to a substrate to produce an array of polynucleotides.]

<u>d) applying target solutions comprising the amplification products to one or more substrates, wherein each target solution is applied to a distinct location on one substrate and/or target solutions are applied to different substrates that are combined to produce an array of polynucleotides.</u>

2. (Canceled) The method of Claim 1 additionally comprising applying the target solutions to one or more substrates, wherein each target solution is applied to a distinct location on one substrate

and/or target solutions are applied to different substrates that are combined to produce an array of polynucleotides.

3. The method of Claim 1 wherein the double-stranded polynucleotide fragments are derived from a polynucleotide library.

4. The method of Claim 3 wherein the polynucleotide library is a genomic DNA library.

5. The method of Claim 3 wherein the polynucleotide library is a cDNA library.

6. (Amended) The method of Claim 3 wherein the double-stranded polynucleotide fragments are derived from YAC, BAC, P1, [or] PAC or cosmid clones.

7. The method of Claim 1 wherein the first polynucleotides each have a complexity of at least about 50 kilobases.

8. The method of Claim 1 wherein the first polynucleotides each have a complexity of at least about 100 kilobases.

9. The method of Claim 7 wherein the first polynucleotides each have a complexity of less than about 500 kilobases.

10. The method of Claim 1 wherein the double-stranded polynucleotide fragments are obtained using one or more restriction endonucleases.

11. The method of Claim 1 wherein the average length of the double-stranded polynucleotide fragments is less than about 5 kilobases.

12. The method of Claim 11 wherein the average length of the double-stranded polynucleotide fragments is less than about 2 kilobases.

13. The method of Claim 11 wherein the average length of the double-stranded polynucleotide fragments is greater than about 100 basepairs.

14. (Amended) The method of Claim 1 [2] wherein the average volume of each target solution applied to the substrate is less than about 2 nanoliters.

15. The method of Claim 14 wherein the average volume of each target solution applied to the substrate is equal to greater than about 0.002 nanoliters.

16. (Amended) The method of Claim 1 [2] wherein the array comprises at least 1000 amplification products in a 1 cm^2 region of substrate.

17. (Amended) The method of Claim 1 [2] wherein the target solutions are robotically spotted on the substrate.

18. (Amended) The method of Claim 1 [2] wherein at least one strand of the adapters includes an amino group.

19. The method of Claim 1 wherein the target solutions comprise dimethyl sulfoxide at a concentration of about 20% by volume.

20. (Amended) An array of polynucleotides that is representative of a plurality of first polynucleotides wherein said array is produced according to the method of Claim 1 [2] and comprises at least 1000 amplification products in a 1 cm^2 region of substrate.

21. (Amended) A plurality of target solutions [prepared according to the method of Claim 3.] useful for forming an array of polynucleotides that is representative of a plurality of first polynucleotides, wherein said target solutions are prepared by a method comprising:

a) providing a plurality of samples of double-stranded polynucleotide fragments, wherein each sample is derived from a first polynucleotide;

b) ligating adapters to each end of the polynucleotide fragments of each sample to produce modified polynucleotide fragments, wherein each adapter comprises a first strand and a second strand, the second strand having a region of substantial complementarity to a region of the first strand;

c) using sequences within the adapters to amplify the modified polynucleotide fragments to produce an amplification product for each sample of polynucleotide fragments, wherein each amplification product is representative of the first polynucleotide corresponding to each sample; and

d) forming target solutions from the amplification products, wherein the target solutions comprise dimethyl sulfoxide at a concentration of about 20% by volume and are suitable for application to a substrate to produce an array of polynucleotides

wherein:

the double-stranded polynucleotide fragments are derived from a polynucleotide library.

22. (Canceled) The plurality of target solutions of Claim 21 wherein the target solutions comprise dimethyl sulfoxide at a concentration of about 20% by volume.

23. The method of Claim 1 wherein each amplification product for each sample is isolated and resuspended to form the target solution for that sample.

24. The plurality of target solutions of Claim 21 wherein each amplification product for each sample is isolated and resuspended to form the target solution for that sample.

25. The method of Claim 1 wherein the first polynucleotides each have a complexity of at least about 20 kilobases.

APPENDIX B

CLAIMS PENDING IN USSN 09/574386 WITH ENTRY OF THIS AMENDMENT

1. (Twice Amended) A method for preparing an array of polynucleotides that is representative of a plurality of first polynucleotides comprising:

a) providing a plurality of samples of double-stranded polynucleotide fragments, wherein each sample is derived from a first polynucleotide;

b) ligating adapters to each end of the polynucleotide fragments of each sample to produce modified polynucleotide fragments, wherein each adapter comprises a first strand and a second strand, the second strand having a region of substantial complementarity to a region of the first strand;

c) using sequences within the adapters to amplify the modified polynucleotide fragments to produce an amplification product for each sample of polynucleotide fragments, wherein each amplification product is representative of the first polynucleotide corresponding to each sample; and

d) applying target solutions comprising the amplification products to one or more substrates, wherein each target solution is applied to a distinct location on one substrate and/or target solutions are applied to different substrates that are combined to produce an array of polynucleotides.

3. The method of Claim 1 wherein the double-stranded polynucleotide fragments are derived from a polynucleotide library.

4. The method of Claim 3 wherein the polynucleotide library is a genomic DNA library.

5. The method of Claim 3 wherein the polynucleotide library is a cDNA library.

6. (Amended) The method of Claim 3 wherein the double-stranded polynucleotide fragments are derived from YAC, BAC, P1, PAC or cosmid clones.

7. The method of Claim 1 wherein the first polynucleotides each have a complexity of at least about 50 kilobases.

8. The method of Claim 1 wherein the first polynucleotides each have a complexity of at least about 100 kilobases.

9. The method of Claim 7 wherein the first polynucleotides each have a complexity of less than about 500 kilobases.

10. The method of Claim 1 wherein the double-stranded polynucleotide fragments are obtained using one or more restriction endonucleases.

11. The method of Claim 1 wherein the average length of the double-stranded polynucleotide fragments is less than about 5 kilobases.

12. The method of Claim 11 wherein the average length of the double-stranded polynucleotide fragments is less than about 2 kilobases.

13. The method of Claim 11 wherein the average length of the double-stranded polynucleotide fragments is greater than about 100 basepairs.

14. (Amended) The method of Claim 1 wherein the average volume of each target solution applied to the substrate is less than about 2 nanoliters.

15. The method of Claim 14 wherein the average volume of each target solution applied to the substrate is equal to greater than about 0.002 nanoliters.

16. (Amended) The method of Claim 1 wherein the array comprises at least 1000 amplification products in a 1 cm^2 region of substrate.

17. (Amended) The method of Claim 1 wherein the target solutions are robotically spotted on the substrate.

18. (Amended) The method of Claim 1 wherein at least one strand of the adapters includes an amino group.

19. The method of Claim 1 wherein the target solutions comprise dimethyl sulfoxide at a concentration of about 20% by volume.

20. (Amended) An array of polynucleotides that is representative of a plurality of first polynucleotides wherein said array is produced according to the method of Claim 1 and comprises at least 1000 amplification products in a 1 cm^2 region of substrate.

21. (Amended) A plurality of target solutions useful for forming an array of polynucleotides that is representative of a plurality of first polynucleotides, wherein said target solutions are prepared by a method comprising:

a) providing a plurality of samples of double-stranded polynucleotide fragments, wherein each sample is derived from a first polynucleotide;

b) ligating adapters to each end of the polynucleotide fragments of each sample to produce modified polynucleotide fragments, wherein each adapter comprises a first strand and a second strand, the second strand having a region of substantial complementarity to a region of the first strand;

c) using sequences within the adapters to amplify the modified polynucleotide fragments to produce an amplification product for each sample of polynucleotide fragments, wherein each amplification product is representative of the first polynucleotide corresponding to each sample; and

d) forming target solutions from the amplification products, wherein the target solutions comprise dimethyl sulfoxide at a concentration of about 20% by volume and are suitable for application to a substrate to produce an array of polynucleotides

wherein:

the double-stranded polynucleotide fragments are derived from a polynucleotide library.

23. The method of Claim 1 wherein each amplification product for each sample is isolated and resuspended to form the target solution for that sample.

24. The plurality of target solutions of Claim 21 wherein each amplification product for each sample is isolated and resuspended to form the target solution for that sample.

25. The method of Claim 1 wherein the first polynucleotides each have a complexity of at least about 20 kilobases.